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BEATA KWASEK*, DARIUSZ BOGDAŁ*

THE USE OF HYALURONIC ACID IN THE TREATMENT OF OSTEOARTHRITIS OF KNEE CARTILAGE

ZASTOSOWANIE KWASU HIALURONOWEGO W LECZENIU CHOROBY ZWYRODNIENIOWEJ STAWU KOLANOWEGO

Abstract

The aim of this paper is to describe the possibilities of the therapeutic use of hyaluronic acid (HA) in the treatment of osteoarthritis of knee cartilage. This work provides an overview of the literature relating to the structure of hyaluronic acid, its properties and important functions of the human body. Moreover, the construction of the articular cartilage in the knee joint, including events leading to its degeneration, is presented. Viscosupplementation is a treatment for osteoarthritis involving several intra-articular injections of HA at specific time intervals. This treatment results in an increased lubricity, viscosity and elasticity of articular cartilage. Since HA is found naturally in the human body, viscosupplementation improves the biomechanical conditions of the joint whilst minimizing the side-effects of treatment. Hyaluronic acid occurs naturally in a linear form, but for many applications, chemical modifications are necessary. Thanks to this property, it remains in the body long enough to produce the desired therapeutic effect. Modification processes, in particular the cross-linking of HA, increase the mechanical properties of knee cartilage. HA is an example of a new kind of tissue engineering scaffold that is bioactive in both full-length and degraded forms. In turn, hydrogel scaffolds with interpenetrating polymeric network (IPN) gels can simulate the structure of the native extracellular matrix of cartilage tissue.

Keywords: *hyaluronic acid, knee cartilage, osteoarthritis, viscosuplementation, interpenetrating polymer network (IPNs), scaffolds*

Streszczenie

Celem badań jest przedstawienie możliwości terapeutycznego zastosowania kwasu hialuronowego w leczeniu choroby zwyrodnieniowej chrząstki stawu kolanowego. Praca zawiera przegląd literatury dotyczący struktury kwasu hialuronowego, jego właściwości oraz ważnych funkcji jakie pełni w ludzkim organizmie, ponadto budowy chrząstki stawowej w stawie kolanowym oraz czynników prowadzących do jej degeneracji. Wiskosuplementacja jest metodą leczenia osteoporozy polegającą na dostawowych wstrzyknięciach tego biopolimeru w określonych odstępach czasu oraz kilkakrotnych powtórzeniach. Leczenie zwiększa lepkość oraz elastyczność chrząstki stawowej, która jest lepiej odżywiana. Wiskosuplementacja poprawia warunki biomechaniczne stawu, co więcej kwas hialuronowy występuje naturalnie w organizmie człowieka, w ten sposób pomaga zminimalizować skutki uboczne leczenia. W naturalnej postaci kwas hialuronowy występuje w postaci liniowej, ale w wielu zastosowaniach konieczna jest jego modyfikacja chemiczna. Dzięki tej właściwości pozostaje w organizmie na tyle długo, aby wywołać pożądany efekt terapeutyczny. Procesy modyfikacji, w szczególności sieciowanie kwasu hialuronowego zwiększa właściwości mechaniczne materiału. Badania potwierdzają, że hydrożelowe rusztowania z wzajemnie przenikającymi się sieciami polimerowymi (IPNs) mogą symulować strukturę natywną macierzy zewnątrzkomórkowej chrząstki.

Słowa kluczowe: *kwas hialuronowy*, *chrząstka kolanowa*, *osteoporoza*, *wiskosuplementacja*, *IPNs*, *rusztowania*

* Prof. Ph.D. Eng. Dariusz Bogdał, M.Sc. Eng. Beata Kwasek, Chair of Biotechnology and Physical Chemistry, Faculty of Chemical Engineering and Technology, Cracow University of Technology.

1. Introduction

Hyaluronic acid (HA) is a polysaccharide composed of repeating disaccharide units containing D-glucuronic acid and N-acetyl-D-glucosamine alternating β-(1-4) and β-(1-3) glycosidic bonds (Fig. 1).

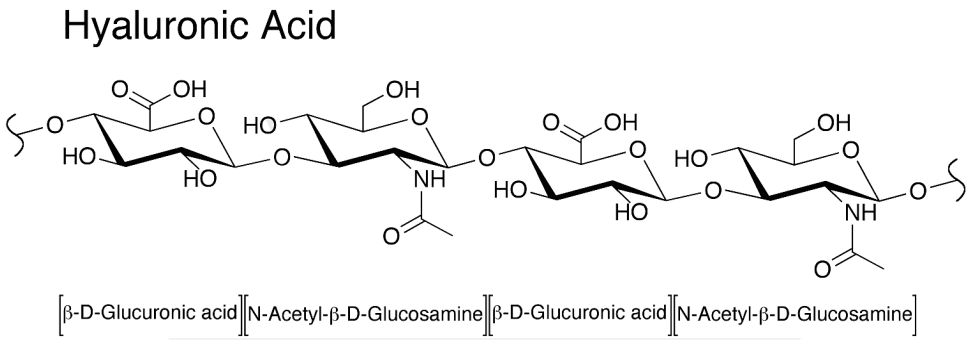

Fig. 1. Structure of hyaluronic acid

HA is a biopolymer which is present in all living organisms and is the largest group of compounds that has the same chemical structure in bacteria and in humans. In animal organisms, hyaluronic acid is synthesized by the cell membrane with hyaluronates synthase, which is involved in connecting alternating glucuronic acid and N-acetylglucosamine molecules. Hyaluronic synthase has a high level of activity, which allows the synthesis of 100 monosaccharide molecules per second under in vitro conditions. There are three different types of hyaluronic synthase: Has 1, Has 2 and Has 3. Has 3 is the most active, able to polymerize large amounts of HA of up to 2×10^5 Da. Hyaluronic acid is present in human skin, cartilage, synovial fluid, the corpus vitreous of the eye, the kidneys, the brain, umbilical cord tissue, urine and serum [1]. Table 1 shows the amount of HA in various parts of the human body.

Table 1

Quantities of hyaluronic acid in human body parts

Locus in the human body	Hyaluronic acid content
an adult weighing 70 kg	15 g HA
dermis	200–500 ug/ml HA
human umbilical cord	4100 ug/ml
synovial fluid of the joint capsule	1400–3600 ug/ml
corpus vitreous of the eye	190- 320 ug/ml
epidermis	100 ug/ml

The amount of hyaluronic acid in the body is dependent upon age – the greatest concentration is in the skin of infants and children, and the smallest, for people over 50 years of age (Fig. 2). The concentration of HA is also dependent on the season – UV

photodegradation is the process leading to photoaging of the skin in the summer, of which the physical symptoms are dry skin and wrinkles. An important property of HA is its high water binding capacity and retention. A single HA molecule is able to bind 200–500 water molecules. Another very important feature of HA is its 'biocompatibility', which means that it does not cause adverse reactions in the body because it naturally occurs in the skin and can be completely absorbed by the body [2]. Figure 2 shows the level of HA in connective tissue.

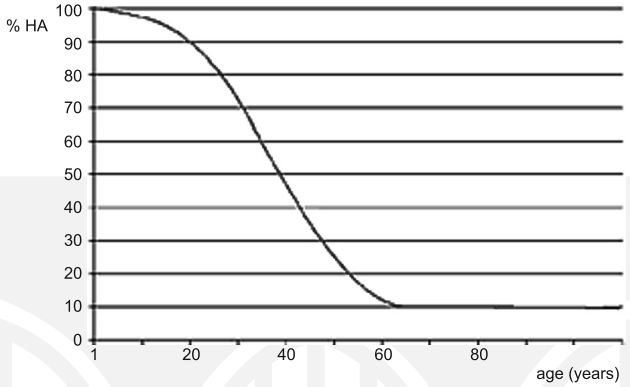

Fig. 2. The level of hyaluronic acid in connective tissue

HA plays an important role in connective tissue, it acts as a matrix. HA helps to keep the skin hydrated and flexibile and protects the retina. HA also affects homeostasis, together with vasopressin, it regulates the reabsorption of water into the spinal cord. An important function of HA is to maintain the connection between the mother and the fetus, the facilitation of the oocyte release during ovulation, the increasing of sperm motility and fertilization efficiency. HA affects a number of processes including the induced expression of cytokines, the stimulation of immune and angiogenic processes. A very important function of HA is its participation in chondrogenesis and providing adequate lubrication of joints which reduces the friction between the moving bones, thereby decreasing the process of osteoarthritis [3].

Articular cartilage is a complex tissue that covers the surfaces in contact with each bone, prevents abrasion and facilitates slippage. Its role is to provide free and accurate movement in the joint [4].

Articular cartilage tissue is alive, resilient, resistant to mechanical stress and has little regenerative capacity. This cartilage is composed of chondrocytes i.e. only cells found in cartilage that produce and maintain the cartilaginous matrix by producing collagen and proteoglycans. In articular cartilage, four basic layers can be distinguished, each with varying amounts of collagen and water (Fig. 3). Zone 1 is called the surface layer and displays the most stiffness. Zone 2 is an intermediate zone, where cells are randomly distributed and have a more spherical shape. Chondrocytes arranged in columns perpendicular to the articular surface are grouped on Zone 3. Zone 4 is the deepest, calcified, and is adjacent to the subchondral bone. Synovial fluid nourishes the articular cartilage. The necessary condition for the proper nutrition of cartilage is the movement and loading of articular surface. Chondrocytes are supplied with oxygen and nutrients from the synovial fluid by diffusion.

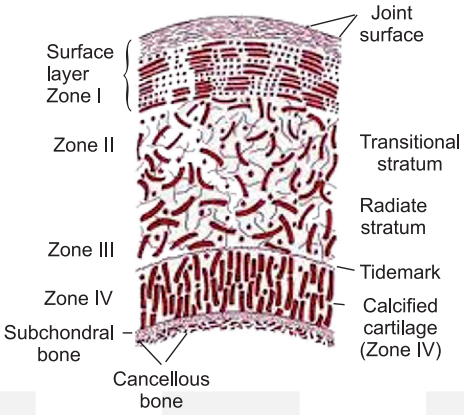

Fig. 3. Articular cartilage matrix

Water makes up about 75% of cartilage – the highest concentration of water is in the deeper layers of the cartilage. The pressure of hydrostatic compression of cartilage causes the extrusion of a small amount of water which forms a layer and reduces friction in the joint.

Other components of the articular cartilage that provides durability and low friction are collagen protein (60% dry weight) and proteoglycan aggregates (30% dry weight). Chondrocytes are sensitive to mechanical stimuli, growth factors, cytokines and receptors. Chondrocytes secrete both the matrix components and the enzymes degrading it. They are responsible for the homeostasis of the cell. Cartilage proteoglycans consist of a protein core to which are attached glycosaminoglycans. This cartilage proteoglycan chondroitin sulfate A, C, and keratan sulfate are important aggrecans capable of binding to HA [5]. Figure 4 shows the appearance of a healthy knee joint in comparison to a degenerate knee joint.

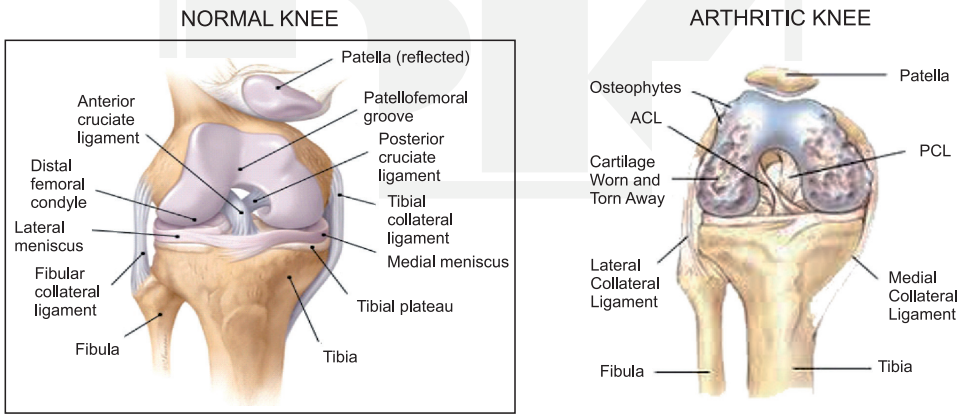

Fig. 4. Healthy knee joint in comparison to degenerate knee joint

Knowledge of the structure and physiology of cartilage allows for understanding the pathomechanisms responsible for the destruction in the course of injury, degenerative

diseases and autoimmune diseases. Degeneration of articular cartilage is a growing problem of civilization. Until recently, the disease was characteristic of old age. Nowadays, more young patients receive medical consultation due to joint pain. The causes of degenerative changes can be: multiple injuries (sports, work); microfractures; chronic stress caused by impaired limb axis; instability or damage to the meniscus; ligamentous instability; rheumatism; obesity [6]. These processes destroy the joints. The disease causes the abnormal changes in synovial fluid, the increased friction of the articular surfaces or the destruction of cartilage. Due to the depth and nature of the damage, articular cartilage is the third most common disease in Central and Eastern Europe (after the ischemic heart disease and vascular diseases of the brain). It usually occurs in the range of 45 to 59 years of age (WHO, 1989), and the other in Western Europe (after the ischemic heart). The prevalence of this condition in the population by age is as follows: under 25 years of age – 4%; under 35 years of age – 5%; over 65 years of age – 70%; in the range 75–79 years – 85%. In Poland, it is estimated that about 8 million people have damage to articular cartilage – 40 % relates to hip and 25% is degeneration of knee joints [8, 9]

Prevention of degradative changes of articular cartilage is possible in early stage. Articular cartilage and synovial fluid cushion the joint. Properties of HA act on smooth movements in all joints, increasing the viscosity of the synovial fluid. Due to the presence of HA, joints are better hydrated, nourished and avoid the release of free radicals.

Research shows that human synoviocytes derived from degenerative arthritis which were incubated with the exogenous HA, synthesize larger amounts of HA and of a higher molecular weight [10].

2. Intra-articular injections of HA

The human body contains approximately 15 grams of pure HA produced by synovial cells. Its defects are increasingly replaced by a synthetic counterpart, introduced by intra--articular injection. Viscosupplementation is a method which consists of intra-articular HA supplementation administered every seven days, and repeated three to five times [11]. This is a common treatment which has been used for over 20 years for osteoarthritis [12–17]. Many studies confirm the effectiveness of viscosupplementation [18–37]. This treatment increases the viscosity and flexibility of synovial fluid [38]. For clinical use, a number of derivatives of HA are available. They are characterized by different molecular weights and viscosities depending upon their source (e.g. rooster combs or hyaluronans secreted by streptococci). Sodium hyaluronan (Hyalgan, Sanofi Synthelabo Inc., New York, NY), and hylan GF-20 (Synvisc, Wyeth-Ayerst Pharmaceuticals, Philadelphia, PA) are available in the United States [29]. Positive effects were observed within 7–14 months of three intra-articular Hylan GF-20 injections using one hundred and fifty-five patients (male and female) with knee osteoarthritis [39]. Hyalgan is a viscous solution of the sodium salt of HA with a molecular weight of between 500.000 and 730.000 Da, it is a highly purified fraction of natural sodium hyaluronate. Synvisc is chemically cross-linked with formaldehyde and vinyl sulfone; its average molecular weight is 6.000.000 Da. Hyalgan has been most commonly administered in a cycle comprising five injections over a 4-week period. More recently, approval has been

given by the Food and Drug Administration (FDA) for a three-injection series. Synvisc is used in a course of one injection weekly for a total of three weeks.

Fig. 5 shows the effect of the Ortovisc preparation for knee pain during 27 weeks of therapy the treatment of osteoarthritis [40].

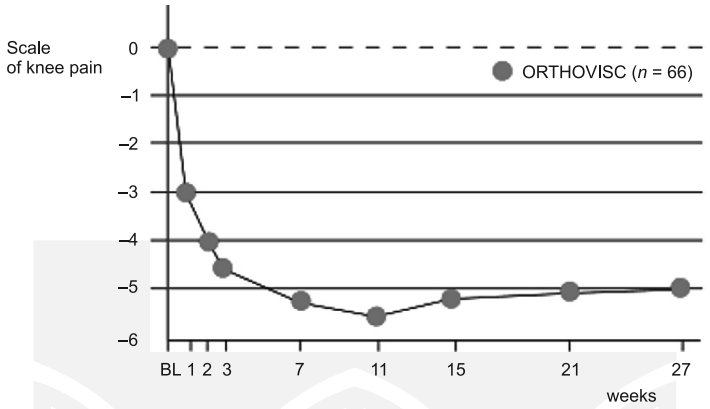

Fig. 5. Orthovisc treatment preparation

The effect of HA on the course of osteoarthritis was identified after clinical trials on patient suffering from this disease. One group of patients was injected with intra-molecular weight hyaluronic acid and the other group receiving a placebo [41, 42]

The study was conducted on 301 patients, who received HA and placebo. Of patients aged over 45 years, 84% were women. The treatment lasted 40 months and involved four cycles of five injections of 2,5 ml 1% solution of HA with a molecular weight of 900 kDa into the knee joint.

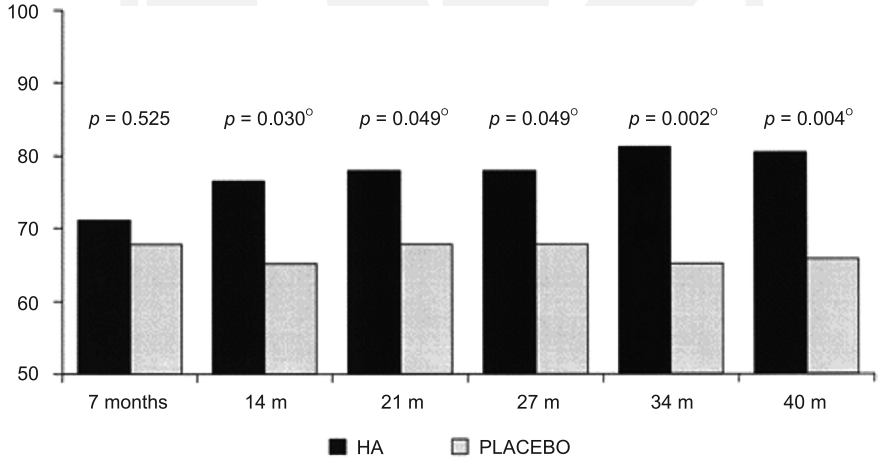

Fig. 6. 40 months of therapy with HA and placebo (Osteoarthritis Research Society International, 2004)

According to the criteria OARSI 2004 – 22% higher response in patients treated with HA (Fig. 6). In general, the clinical results showed that viscosupplementation with hyaluronic acid is most effective in patients aged over 65 years [44–51].

3. Chemical modification of hyaluronic acid

HA is subjected to a process of linear and crosslinking modifications by forming covalent bonds between molecules of HA. Modified HA is less susceptible to chemical and enzymatic degradation, which allows it to remain in the body long enough to produce the desired therapeutic effect [52]. Modification processes, in particular the cross-linking of HA, also enhance the mechanical properties of the material. Chemical modifications typically involve hydroxyl and carboxyl groups of the polymer.

Alkylation of the ammonium salt of hyaluronic acid with an alkyl halide in a solution of DMF leads to the formation of esterified HA biomaterials. Esterification reactions involve carboxylate moieties of the polymer. The degree of esterification affects the size of the hydrophobic patches and forms a stable, rigid chain network polymer more resistant to enzymatic degradation under physiological conditions. Esters of HA are the most utilized derivatized HA products.

These products are applied to the growth of chondrocytes and bone marrow derived mesenchymal cells for the repair of cartilage and bone defects.

Crosslinking HA is necessary to obtain implantable hydrogel with controlled degradability and mechanical properties. Crosslinking hydrogels is the most common modification of HA [52]. Crosslinking (binding a polymer with other polymers) of the hyaluronic acid results in its stability and extends the natural degradation [53]. Degradation of crosslinked HA in the body lasts for several months, whereas the degradation of non-crosslinked HA takes a few hours. Viscosity of the crosslinked HA increases and depends on the degree of cross-linking between the chains of HA. Thanks to this property remain in the body long enough to produce the desired therapeutic effect.

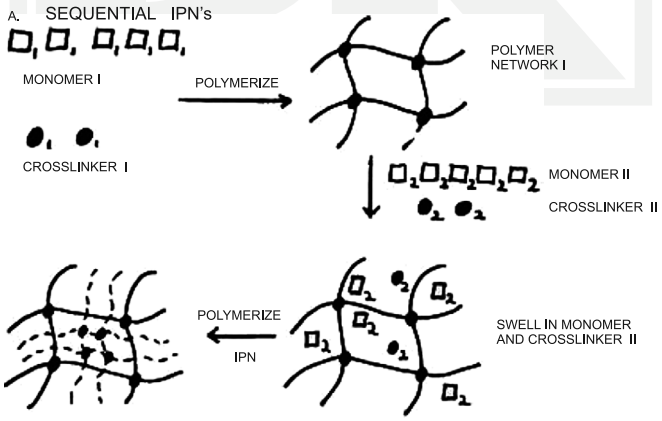

Fig. 7. Basic synthesis methods for IPNs.

An interpenetrating polymer network, IPN, is defined as a blend of two or more polymers in a network form. Figure 7 shows the synthesis method of IPNs.

Hydrogels of collagen/chondroitin sulfate/hyaluronan interpenetrating polymer network were used for cartilage tissue engineering [54].

The task of tissue engineering is the development of substitutes for damaged tissue by creating three-dimensional scaffolds with living cells and bioactive molecules, which allow the proliferation and differentiation of cells. These structures can be created by seeding cells within the scaffold or by the injection of cells into the damaged tissue. Injectable scaffolds have been used to repair articular cartilage. Injectable biocompatible and biodegradable scaffolds derived from chitosan and oxidized hyaluronic acid [55] and injectable hydrogel made from methacrylated glycol chitosan (MeGC) and photocrosslinking HA with a ryboflavin photoinitiator under visible light were found to be very good options for cartilage repair [56].

Lisognoli investigated human mesenchymal stromal (MSCs) cells in a hyaluronian-based polymer scaffold (Hyaff ®-11) by electron microscopy techniques, immunohistochemistry and real time PCR. There was a significant proliferation of cells TGFβTGFβ MSCs and increased expression of collagen type II, IX within 21 days.

Hyaff-11 was also used as scaffolding for cartilage defects [57–61]. Autocrosslinked polysaccharide (ACP) polymers are inter and intra-molecular esters of hyaluronan (HA) where part of the carboxyl group is esterified with hydroxyl groups of the same or different molecules of polysaccharides. The autocross-linked polymer (ACPTM, Fidia) is used as a scaffold for the repair of tissue defects. This biomaterial has been used as a scaffold and showed faster regeneration of tissue defects than Hyaff 11 [62].

Scaffold based on HA is a kind of bioactive tissue engineering with a specific interaction between the scaffold and growing cells by cell receptors (CD44, RHAMM, ICAM-1) for the growth and repair of tissues. Chung IPNs made with sodium hyaluronic acid/sodium alginate (HA/SA) scaffold, where HA and SA were cross-linked with diglycidyl ether of polyethylene glycol (PEGDG) and calcium chloride. The study used rabbit chondrocytes which are seeded in a HA/SA scaffold. Researchers noticed an increase in the quantity of DNA in chondrocytes after 21 days which confirmed their proliferation and increased the quantity of collagen type II. These studies demonstrated that porous HA/SA scaffolds

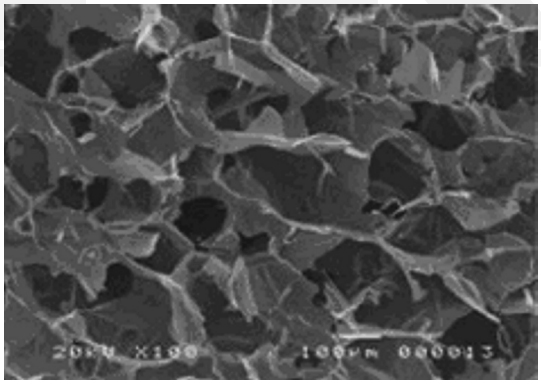

Fig. 8. SEM image of fabricated HA70/SA30.

may be used for three-dimensional culture of chondrocytes. A low pore size HA/SA (70/30) scaffold about 200 μm is preferable for durability and chondrocyte differentiation (Fig. 8) [63].

Hydrogels made from the crosslinked polymers with a high water content can provide local delivery of therapeutic agents, because of this benefits are called “intelligent materials”.

4. Conclusions

Intra-articular injections of HA provides hope for improvements in the treatment of patients suffering from arthritis, where the concentration of hyaluronic acid conditioning the viscoelastic properties of synovial fluid and proper movement in the joint is reduced. Viscosupplementation is a treatment for osteoarthritis involving the intra-articular injection of HA in appropriate quantities and time intervals. The number publications presented in this work confirms of analgesic, anti-inflammatory and articular tissue protect functions of HA. Viscosupplementation is an alternative method for treating arthritis. Injectable, biodegradable scaffolds based on HA in the form of hydrogels are important biomaterials for tissue engineering and drug delivery. Hydrogels derived from HA are ideal scaffolds as they resemble the extracellular matrices of tissues comprised of various glycosaminoglycans (GAGs).

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